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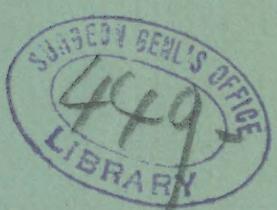
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THE HISTOLOGICAL CHANGES IN EXPERIMENTAL DIPHTHERIA.¹

PRELIMINARY COMMUNICATION.

By WILLIAM H. WELCH, M. D., *Professor of Pathology*, and
SIMON FLEXNER, M. D., *Fellow in Pathology*.

(*From the Pathological Laboratory of the Johns Hopkins University and Hospital.*)



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The well-known observations of Oertel concerning peculiar histological changes in internal organs in human diphtheria, make it important to determine whether similar alterations occur in animals inoculated with the Klebs-Löffler bacillus of diphtheria. If such changes are found in the latter cases, they add valuable proof to the demonstration that the experimental is essentially identical with human diphtheria.

Microscopical changes in the internal organs, especially in the liver and kidney, in cases of experimental diphtheria have been observed by Löffler, Klein, Beck, and others. The fullest description thus far given of such changes is by Babes,² whose investigations on this point relate chiefly, also, to the liver and kidneys of rabbits. The changes in the liver observed by Babes varied in different cases. The principal lesions in this organ noted by him are swelling and degeneration of the liver cells, as well as proliferation of these cells, accumulation of leucocytes, often with fragmented nuclei (which, in some cases at least, he regards as evidence of proliferation), and of hyaline material and yellowish granular substance in the blood-vessels, and swelling and proliferation of the endothelial cells of the capillaries. In the kidneys he observed parenchymatous swelling of the epithelial cells, proliferation and desquamation of these, as well as of endothelial cells, and hyaline masses in the blood-vessels. In the spleen he found hy-

¹ Reported to the Johns Hopkins Hospital Medical Society, May, 1891.

² Babes, *Virchow's Archiv.*, Bd. 119, p. 468.



perplasia of the lymphatic apparatus, and in one case he speaks of numerous nuclear fragments in the venous lacunae.

Our study has been conducted with the view of ascertaining the histological changes in all of the organs of animals dead of experimental diphtheria. In the course of our work we have used guinea pigs, rabbits and kittens.

At the present time our experiments are not so complete as we wish to make them, but our results have been so uniform that we feel justified in presenting a brief preliminary account of them to the Society.

The cultures used in our work were obtained from undoubted cases of primary diphtheria occurring in Baltimore, and they were in all cases pure cultures of the Klebs-Löffler bacillus.

The inoculations were made into the subcutaneous tissue of the belly wall, a site being chosen midway between the axillary and inguinal regions. There was one exception to this rule, in the case of a kitten on which tracheotomy was performed and the inoculation made into the trachea.

Death occurred in the animals experimented upon in the following lengths of time:

Guinea pigs—shortest period, 38 hours; longest period, 5 days.

Rabbits—shortest period, 48 hours; longest period, 8½ days.

Kittens—shortest period, 43 hours; longest period, 5½ days.

The *gross anatomical lesions* which we observed corresponded for the most part with those described by other investigators. At the seat of inoculation was a grayish, necrotic, pseudo-membranous focus, and surrounding this a deep red zone of varying size; underlying it the muscle of the belly wall was greatly congested.

The subcutaneous tissue on the side of inoculation was oedematous beginning at the seat of inoculation and extending over the entire side, often into the neck, and in some cases beyond the median line to the opposite side. The axillary and inguinal regions and the place immediately around the seat of inoculation were principally affected, and the oedema often assumed an exquisite gelatinous appearance.

In some cases this oedema was composed of pure yellowish serum, and in others it consisted of serum stained with blood. The subcutaneous lymph glands of the axillary, inguinal and cervical regions were hemorrhagic and swollen. The swelling affected the glands most on the side of inoculation; but the glands of the opposite side were reddened and sometimes en-

larged. We would call attention to the ease with which moderate glandular enlargement in such small animals may be overlooked, unless the very small size of the normal glands be kept in mind.

The cavities of the body showed alterations. In some cases the peritoneal cavity contained an excess of fluid—as much, sometimes, as five or six cc. Both the visceral and parietal layers of peritoneum were much injected, and ecchymoses were present in some cases. The mesenteric glands and retro-peritoneal glands were enlarged, and sometimes distinctly reddened.

The organs were not unaffected. The liver was congested ; at times evidently fatty. In guinea-pigs, especially, the liver showed numerous macroscopic dots and lines on the surface and penetrating the substance of the organ. In a less degree this was true of kittens also. These dots varied in size from a pin-point to a pin-head ; sometimes they were even larger. The lines were two mm. in length, or less. They appeared white, and did not project above the surface of the capsule.

Though the spleen was not perceptibly enlarged in all cases, yet in some it was markedly enlarged. There was considerable variation in this respect. Its color varied : in guinea-pigs there was no striking alteration, while in kittens the spleen was dark, often bluish black.

The kidneys were moist and hyperaemic.

The adrenals always showed an intense congestion, and in many instances were hemorrhagic.

The intestinal lymphatic apparatus showed interesting alterations. The agminated glands of the caecum and the ileum in guinea-pigs and kittens were abnormally prominent. They projected above the surface of the bowel, and exhibited a number of whitish points imbedded in a grayish material.

The pleural cavity contained in many cases an excess of fluid, amounting, at times, to three or four cc. The mediastinal glands were swollen and reddened, and the bronchial glands likewise. The heart appeared normal, while the lungs sometimes showed areas of congestion, and even of consolidation. The deep glands of the neck were often reddened, and the thyroids were uniformly congested, sometimes hemorrhagic.

The *microscopical study* of the organs was made in the fresh state by means of frozen sections, and, again, after hardening in different ways ; alcohol, Flemming's solution and Mueller's fluid having been used. The hardened tissues were stained with various

aniline dyes, hematoxylin and carmine. The tissues hardened in alcohol and stained with fuchsin or methylene blue, gave, as a rule, the most satisfactory results, other methods being useful for developing certain points.

Seat of Inoculation.—Bacilli of diphtheria were found regularly in the seat of inoculation. They were present in the gray necrotic-looking focus both free and within leucocytes. They may be absent from the oedematous fluid at a distance from this focus. Many of the leucocytes showed a fragmentation of their nuclei. Sections made from the seat of inoculation showed the bacilli in great numbers. The sections stained by means of Weigert's fibrin stain exhibited in a striking manner the bacilli and the fibrin.

The local action of the bacilli is of the most intense character. There is emigration and great destruction of leucocytes shown by disintegration of their nuclei; the fixed cells of the part have undergone a similar fragmentation, nuclei of connective tissue and muscle have succumbed, and leucocytes have wandered into these areas, many of the latter being destroyed also. There would appear to have been a proliferation of muscle nuclei in places not all of which have become fragmented.

Lymphatic Apparatus.—The lymph glands of the axillary and the inguinal regions were greatly affected. The changes in these structures are very typical.

There are hemorrhages under the capsule and into the substance of the gland. The blood-vessels here as elsewhere in the body contain a greatly increased number of leucocytes.

The cells of the gland are the seat of great changes. The principal lesions are in the lymph follicles, the lymph cords and lymph sinuses being also affected, though in less degree. Different follicles are affected in different degrees, and there are variations in different animals even of the same species. But in no instances were the lesions entirely absent.

The lesions consist of a marked alteration in the number, character, size, staining capacity and configuration of the nuclei making up the parts affected. The cell bodies are altered also, and an increased number of cells differing from the lymphoid type are found. The first thing that attracts attention is the unusual number of deeply-staining bodies in the tissue. These bodies are observed to vary in size and shape, and under a sufficiently high-power some of them are recognized as nuclear figures. They are

usually, however, globular, and under a magnifying power of four hundred range from fine dust-like particles to larger particles, appearing with this power the size of a pin's head. The finer particles are often aggregated into larger globular masses, which are now free, and now enclosed in cells.

There are, again, deeply-staining particles present which show decided bizarre forms. Imperfect crescents, flask-shaped, bladder-like, whetstone, angular, and dumb-bell forms are more or less common. Occasionally, nuclei appear as if one end were drawn out and constricted into a ball-like protuberance that is being pinched off. The globular particles are, at times, grouped together with the bizarre forms into larger masses; what particularly distinguishes these bodies from the normal nuclei which remain is the intensity with which they stain.

All the chromatin particles, as before mentioned, are not within cells; indeed, as a rule, they do not occur in cells, though the number within cells varies considerably. In some glands much of this material is contained within globular cells several times larger than the lymphoid cells. In some sections these cells are observed to be present in considerable numbers, partly devoid of stained particles, or nearly so, but usually they are full of the nuclear detritus.

In certain spots there is almost an absence of stained cells and particles. In these places it is possible to distinguish outlines of cells, then a finer granular, somewhat refractive, at times reticulated material, and here and there a deeply-stained particle. But there are always a few cells remaining that stain more or less, and among these are round cells, larger than the lymphoid cells. This portion stains with Weigert's fibrin stain in such a manner as to indicate that the granular material is largely made of fibrin or of a substance allied to fibrin.

In the lymph sinuses, especially, occurred a considerable number of round or slightly oblong cells, quite colorless in appearance, containing bodies which resemble in a striking manner red-blood-corpuscles, and these cells remind one of the red-blood-corpuscle carrying-cells of the typhoid spleen. In alcohol preparations it was impossible to determine the exact nature of the contents of these cells. Similar cells were found in the mesenteric glands and the spleen; in osmic acid preparations, on the other hand, they are shown to be red corpuscles.

An alteration similar to that just described is found throughout

the lymphatic structures of the body: in the spleen, mesenteric glands, retro-peritoneal glands, intestinal lymphatic apparatus (Peyer's patches, solitary follicles, and diffused lymphatic tissue), the bronchial glands, mediastinal glands, and cervical glands. The only variation is one of degree. As far as our study has gone every gland examined has been more or less affected. The spleen is often very rich in nuclear fragments and foci of coagulation necrosis.

The cells of the *intestinal villi* in guinea pigs and rabbits and the epithelium of the intestinal mucous membrane were involved. The lesions in these structures showed a like tendency to vary in intensity. The most striking changes consisted in a fragmentation of the nuclei of cells in the villi, especially of those surrounding the central vessels (lacteals or blood-vessels), a disappearance of a large number of cells, and the presence of large round cells, similar to those described in the parts of the lymph glands most affected. These larger cells often showed a very slight staining power and shadows of cells were not uncommon. The nuclei of the epithelium were distinctly and extensively fragmented. Nuclear figures were to be seen and saprophytic bacteria were found in the necrotic tissue. These fragmented nuclei partook of the same characters as those already described and exhibited the same intense affinity for staining agents.

The Liver.—On frozen sections the dots and lines resolve themselves into smaller and larger masses of highly refractive cells, usually devoid of nuclei and retaining the outlines of liver cells. Reagents do not affect them to any great extent; acetic acid causes them to swell somewhat and to become slightly less refractive. These cells are hyaline and represent foci of dead liver cells. Besides the groups which were visible to the naked eye as lines and dots there are small groups and single cells which show the same change.

Sections of the hardened organ were studied. Those from alcohol were stained in methylene blue and eosine. By this method the areas of dead liver cells stand out clearly in red. The cells generally have well stained blue nuclei and lightly stained red cell bodies. But the hyaline cells are intensely red, either devoid of nuclei or, when a nucleus is present, it is manifestly altered. It is shrunken, often irregular in shape, and it stains differently from the nuclei generally.

Leucocytes are commonly found in such foci of dead liver cells. They are between the hyaline cells and sometimes apparently

within them. There are fragments of nuclei in these places, some of which at least have been derived from the leucocytes which have wandered in.

An important change could be made out in these livers in connection with the central veins of certain lobules. Hemorrhages under the capsule were quite common, and in these lobules deep-seated hemorrhages into the tissue had taken place. These varied greatly in size, some being very small while others were quite large, involving the greater part of the lobules. It was observed that such intra-lobular hemorrhages originated in the central veins, and seemed to be due to a breach of continuity in their walls. For, when they were small a minute defect could sometimes be seen, and where they were larger a considerable rent was present in the vein wall through which the blood had escaped. The walls of these veins were quite refractive in the fresh state, and gave, after hardening, a quite characteristic staining for hyaline with picric acid.

This focal death of liver cells just described was observed in guinea-pigs and kittens especially. In rabbits, in one case in particular, there was an extensive diffuse degeneration of liver tissue. The liver was yellowish and evidently fatty, and on frozen sections it proved to be the seat of a most extensive fatty degeneration. The cells, after the tissue had been hardened in alcohol, stained very imperfectly, many not at all. The nuclei of the liver cells were in many places in a state of disintegration or fragmentation ; a few, of division. The cells throughout entire lobules were affected. Leucocytes were numerous, both in the tissues and in the vessels, and many of those in the latter were fragmented.

The Kidneys.—Fresh frozen sections showed in guinea-pigs and rabbits fatty changes in the epithelium of the tubes and glomeruli. In kittens, owing to the large amount of fat in the epithelium normally, it is not easy to make out a pathological increase. Yet there would appear to be an absence of fat in the epithelium of the glomeruli and collecting tubes in the cortex under normal conditions. In some of our cases these structures contained minute fat drops. A more important change is a hyaline alteration of the glomerular capillaries and the smaller arteries. This was noticed in kittens in particular ; and it often affected a few loops of a glomerulus, without involving the entire mass of capillaries. Hyaline substance was found completely filling the lumen of some capillaries.

Hardened and stained sections showed besides the hyaline change just mentioned fragmentation of the nuclei of the glomerular and tubal epithelium on a small scale, and the presence of minute deeply-staining round bodies between and in the tubal epithelium.

Adrenals.—These were generally congested, hemorrhages were often present, and in two cases the medullary cells were distinctly hyaline.

Lungs.—There were often hemorrhages under the pleura. The blood vessels of larger size are distended with blood, and the number of leucocytes in them increased. The capillaries in the walls of the alveoli are distended with blood likewise, and there are hemorrhages into the alveoli. There is an exudation into some of them consisting of leucocytes and fibrin; but the leucocytes are few in number. There are many large cells present having a round nucleus of considerable size which stains quite deeply, but not solidly, with fuchsin. These cells are present in the areas of consolidation, and they are found also in considerable number in the nodes of the alveoli, that is, the points at which several of them come together. They are probably derived from the epithelial cells of these structures.

Fragmentation of nuclei is not a prominent feature in the lungs. It does occur, but not extensively. It was noticed in the areas of exudation, where it affects the nuclei of the large round cells as well as the leucocytes. Then it is seen in the epithelial lining of the larger bronchi, and in those bronchi that contain leucocytes fragments of nuclei are present in their lumina amid the exudation.

The *heart* was the seat of a fatty degeneration, more or less intense, in nearly every case. In many of them every muscle fibre seemed to have undergone a minute fatty metamorphosis. In others the process was less general.

Another change was noticed in the nuclei of the *muscle* fibres in certain cases. It consisted of a fragmentation similar to that in other parts.

The muscles adjoining the lymphatic glands of the axillary and inguinal regions showed degenerative changes. There was necrosis of muscle fibres and wandering in of large numbers of leucocytes, a myositis being the result.

Cultures were made from the blood, liver, kidney, and spleen of these animals with negative results in every instance.

